Published in final edited form as:

Neuroscience. 2014 June 20; 271: 23–34. doi:10.1016/j.neuroscience.2014.04.024.

Ultrastructural localization of tyrosine hydroxylase in tree shrew nucleus accumbens core and shell

Lesley A. McCollum^{*} and Rosalinda C. Roberts

Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, Alabama 35294 USA

Abstract

Many behavioral, physiological, and anatomical studies utilize animal models to investigate human striatal pathologies. Although commonly used, rodent striatum may not present the optimal animal model for certain studies due to a lesser morphological complexity than that of non-human primates, which are increasingly restricted in research. As an alternative, the tree shrew could provide a beneficial animal model for studies of the striatum. The gross morphology of the tree shrew striatum resembles that of primates, with separation of the caudate and putamen by the internal capsule. The neurochemical anatomy of the ventral striatum, specifically the nucleus accumbens, has never been examined. This major region of the limbic system plays a role in normal physiological functioning and is also an area of interest for human striatal disorders. The current study uses immunohistochemistry of calbindin and tyrosine hydroxylase (TH) to determine the ultrastructural organization of the nucleus accumbens core and shell of the tree shrew (*Tupaia glis belangeri*). Stereology was used to quantify the ultrastructural localization of TH, which displays weaker immunoreactivity in the core and denser immunoreactivity in the shell. In both regions, synapses with TH-immunoreactive axon terminals were primarily symmetric and showed no preference for targeting dendrites versus dendritic spines. The results were compared to previous ultrastructural studies of TH and dopamine in rat and monkey nucleus accumbens. Tree shrew and monkey show no preference for the postsynaptic target in the shell, in contrast to rats which show a preference for synapsing with dendrites. Tree shrews have a ratio of asymmetric to symmetric synapses formed by TH-immunoreactive terminals that is intermediate between rats and monkey. The findings from this study support the tree shrew as an alternative model for studies of human striatal pathologies.

Keywords

electron microscopy; dopamine; striatum; immunohistochemistry; ultrastructure

© 2014 IBRO. Published by Elsevier Ltd. All rights reserved

^{*}Correspondence to: Lesley A. McCollum, University of Alabama at Birmingham, 841 Sparks Center, 1720 2nd Ave South, Birmingham, AL 35294. Phone: 205-934-1858 Fax: 205-996-9377 lbryant@uab.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The nucleus accumbens (NAcc) is a region of the ventral striatum that plays an important role in limbic-related functioning, such as reward and motivation, emotional processing, and goal-oriented behaviors (see Groenewegen, 2007). Two distinct subregions of the NAcc, the core and shell, have been described in rodent (Záborszky et al, 1985), monkey (Meredith et al, 1996; Brauer et al, 2000), and human NAcc (Meredith et al, 1996). These subregions can be distinguished based on neurochemical, anatomical, and functional characteristics; the core is more similar morphologically to the dorsal striatum, while the shell has been associated with the extended amygdala (Meredith et al, 1992; Zahm and Brog, 1992; Brog et al, 1993; Heimer et al, 1997; Zahm, 1999; Prensa et al, 2003). The NAcc receives excitatory input from numerous brain regions including the medial prefrontal cortex, anterior cingulate cortex, thalamus, hippocampus, and amygdala (Groenewegen et al, 1987; Berendse et al, 1992; Brog et al, 1993; Kunishio and Haber, 1994; Giménez-Amaya et al, 1995; Haber et al, 1995; Wright and Groenewegen, 1995). GABAergic neurons of the NAcc send projections primarily to the ventral pallidum and substantia nigra (Haber et al, 1990; Nauta et al, 1978) which in turn project to the thalamus and dorsal striatum, respectively (Gerfen et al, 1987; Groenewegen et al, 1993; Groenewegen and Berendse, 1994; Deniau et al, 1994). Thus, through these pathways, the NAcc plays a critical role in thalamo-cortical pathways (Alexander et al, 1986; Deniau et al, 1994; Groenewegen et al, 1996) as well as in the modulation of dopaminergic input to the dorsal striatum (Groenewegen et al, 1996; Haber et al, 2000).

In addition to the input discussed above, the NAcc receives abundant dopaminergic input from the ventral tegmental area and substantia nigra (Gerfen et al, 1987; Lynd-Balta and Haber, 1994), which plays a key role in the modulation of neuronal firing within the NAcc (for reviews see Nicola et al, 2000; O'Donnell et al, 1999). Normal dopaminergic innervation is critical for processing in the striatum which is evident in the numerous disorders that have been associated with striatal dopamine (DA) dysfunction, including Parkinson's disease, addiction, and schizophrenia (Albin et al 1989; Koob and Nestler, 1997; Robbins and Everitt, 2002; Perez-Costas et al, 2010).

The use of animal models has been instrumental in understanding these and other striatal disorders. Rodents are most commonly used as animal models in research, and while there are many benefits to using rodents such as cost and efficiency, they have specific disadvantages when it comes to studying human striatal disorders. The major anatomical difference in the basal ganglia is that rodents lack an internal capsule, the tract of white matter that extends through and divides the human striatum. Thus, the rodent striatum does not have a separate caudate and putamen as is present in the human dorsal striatum. This poses a limitation to the knowledge that can be gained from this model, as studies in human patients show that these subregions are differentially affected in striatal disorders. Within the ventral striatum, core and shell subregions have been described across species, as discussed above, however a third subregion in the rodent NAcc, the rostral pole, has not been identified in primates (Ikemoto et al, 1995; Meredith et al, 1996). Also within the ventral striatum, variation in neurochemical distribution (Meredith et al, 1996) and in afferent and efferent projections with regions including the thalamus, midbrain, and globus pallidus exist between rodents and primates (Haber et al, 1990; Lynd-Balta and Haber, 1994; Gimenez-Amaya et al, 1995). Further, ultrastructural studies in rodent and primate NAcc report

diverging patterns in synaptic connectivity, particularly in dopaminergic synapse organization (Arluison et al, 1984; Bouyer et al, 1984; Voorn et al, 1986; Ikemoto et al, 1996b). Non-human primates are indeed a superior model as the anatomical features of the non-human primate brain more accurately represent that of the human brain, however, non-human primate studies are costly and increasingly restricted in research. These issues point to the need for a less controversial animal model than non-human primates with anatomical features more accurately representing the human striatum.

An animal that offers the potential to meet these needs is the common tree shrew (*Tupaia glis belangeri*), belonging to its own order Scandentia. Advances in molecular phylogenetics have classified this diurnal species as one of the closest living relatives to primates (Adkins and Honeycutt, 1991; Liu et al, 2001; Murphy et al, 2001; Janecka et al, 2007). While tree shrews have been established as models for research in depression, myopia, and hepatitis C infection (for reviews see Fuchs, 2005; Norton, 1990; MacArthur et al, 2012, respectively), very few studies have investigated their brain anatomy (Mijnster et al, 1999; Rice et al, 2011). In the interest of studying human striatal disorders, the tree shrew striatum exhibits the complex anatomy that is lacking in the rodent striatum: the separation of the caudate and putamen via the internal capsule. A recent study characterizing the neurochemical anatomy of the dorsal striatum showed similarities between the tree shrew and primate brain in the distribution of parvalbumin, calbindin, and tyrosine hydroxylase, as well as in cytoarchitectural organization (Rice et al, 2011). Further, Mijnster and colleagues (Mijnster et al, 1999) mapped D1- and D2-type dopamine receptor binding patterns in tree shrew striatum, and discussed how the patterns in tree shrew are similar to those in primate, and diverge from those in rodent (Mijnster et al, 1999). The similarity of the tree shrew dorsal striatum to that of primates, as well as their close phylogenetic relationship to one another, elicits the question of how the ultrastructural organization of the tree shrew compares to that of primates, as opposed to rodents. More specifically, considering 1) the relevance of DA in numerous human striatal disorders and 2) the species differences in synaptic DA organization in the NAcc of rodents and primates, it is of key interest to determine if the tree shrew synaptic DA organization provides a better model for that of primates than is available with rodents.

In efforts to further characterize the anatomy of the tree shrew striatum, the present study utilizes immunohistochemistry of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, and electron microscopy to examine the ultrastructural organization of the DA system in the tree shrew NAcc. Results of the current study were compared to previous ultrastructural studies of TH and DA in rodent and primate NAcc to assess its similarities with current striatal models and consider the validity of the tree shrew as an anatomical model for the human striatum. These data have been presented in preliminary form (McCollum and Roberts, 2012).

Experimental procedures

2.1 Animals

Tissue from 6 tree shrews (*T. glis belangeri*) was used for this study (ages: 3 months-5 years; male: $n=2$, female: $n=4$). Animals were housed and maintained at the University of

Alabama at Birmingham under IACUC approval and oversight (APN 120208727). Prior to tissue acquisition, animals had been deeply anesthetized with a ketamine/xylazine mixture and perfused intracardially with a 0.9% saline solution followed by 1% glutaraldehyde, 4% paraformaldehyde in 0.1 M phosphate buffer (PB) solution. Due to poor demarcation of ultrastructure, one animal (male) was excluded from ultrastructural analyses of synapse types and was only used for qualitative observations and quantification of total TH-immunoreactive (–IR) terminals.

2.2 Immunohistochemistry

The tissue was sectioned at a thickness of 40 μ m with a Vibratome (HM 650V microtome, Thermo Scientific) in six series. Two series of free-floating sections were processed for the immunohistochemical localization of one the following primary antibodies: mouse monoclonal anti-tyrosine hydroxylase (Millipore MAB5280; dilution 1:1000) or mouse monoclonal anti-calbindin (Sigma C9848; dilution 1:2000). For both primary antibodies, the secondary antibody used was biotinylated horse anti-mouse IgG (Vector Laboratories, dilution 1:400). All antibodies were prepared in 3% normal horse serum in phosphate buffered saline (PBS). The sections were incubated in a 1% sodium borohydride in PBS solution for 15 minutes. After rinsing, the sections were incubated in 10% normal horse serum in 0.1 M PB for 1 hour, followed by the primary antibody for 20 hours. Sections were then rinsed and incubated in secondary antibody for 45 minutes, and rinsed. The sections were then incubated for 45 minutes with the avidin-biotin complex (ABC standard kit, Vector Laboratories) based on methods of Hsu et al. (1981), and rinsed. To visualize the reaction product, sections were subsequently incubated in 3, 3'-diaminobenzidine (DAB, 10 mg diaminobenzidine, 15 ml PB, 12 μ L 0.03% hydrogen peroxide; Vector Laboratories, SK-4100) for 2–7 minutes. All rinses consisted of four 5-minute washes in PBS. All steps were done at room temperature. Specificity of the staining was verified by omitting the primary antibody, but otherwise performing an identical protocol. When the primary antibody was omitted, staining was absent. Calbindinstained sections were mounted onto glass slides and coverslipped for use as a reference of the core-shell boundaries (Fig. 1).

2.3 Electron Microscopy

Sections stained for TH were flat embedded for electron microscopic analysis using standard techniques. The sections were rinsed two times in PB for 5 minutes each, immersed in 1% osmium tetroxide in 0.1 M PB at room temperature in the dark for 1 hour, rinsed four times for 5 minutes each in PB, then dehydrated at room temperature in the dark in increasing concentrations of EtOH. Following dehydrations, the tissue was stained en bloc in a 1% uranyl acetate solution in 70% EtOH for 1 hour for contrast, and then rinsed in 70% EtOH two times for 5 minutes each. The tissue was dehydrated in increasing concentrations of EtOH, followed by 100% propylene oxide, then embedded in epon resins, and heated at 60°C for 72 hours.

Regions from the NAcc core and shell were blocked for further processing using the calbindin-stained sections as a guide. The dorsomedial-most region of the NAcc near the lateral ventricle was not sampled to avoid potential confounds with the extended amygdala, particularly in the caudomedial shell which has been described as a transitional zone into the

lateral bed nucleus of the stria terminalis (see Heimer et al, 1997). 2–3 blocks per animal, at least 240 μm apart rostrocaudally, were used to obtain semithin sections. These sections (250 nm thickness) were collected using a Leica EM UC6 ultramicrotome, mounted on glass slides, stained with toluidine blue and coverslipped for reference. Serial ultrathin sections (90 nm thickness) from each block were mounted on Formvar-coated copper grids, and photographed at 80 kV on a Hitachi 87650 transmission electron microscope using a Hamamatsu ORCA-HR digital camera. In order to photograph a large field of neuropil, four-by-two montages of individual overlapping digital micrographs were taken and stitched together using PanaVue ImageAssembler 3. Electron micrographs were taken from superficial regions of the embedded tissue sections to ensure even antibody penetration across sections, and away from edges to avoid potential staining artifact. Using anatomical landmarks to locate the region of interest, the same area of neuropil in each serial section was photographed at a magnification of 15,000 $\times$.

2.4 Data Collection and Statistical Analyses

To determine the number of synapses in the neuropil, serial sections were analyzed using the disector technique (Sterio, 1984; Geinisman et al., 1996) as described in Perez-Costas et al. (2007). An average of 7 consecutive sections per block was used as disector reference sections for analysis, yielding a total of 144 sections analyzed for the core and shell. The average sampling volume was 321.2 μm^3 per block. Volumes for each block were obtained using the following equation: $\text{Volume} = N \times 0.09 \times \text{Area}$, where N is the number of disector reference sections used for the quantitative analysis, 0.09 μm is the thickness of each ultrathin section, and Area is the average area of the photographed region (measured using ImageJ). All synapses in this study were identified by both authors using Adobe Photoshop. Micrographs were cropped and adjusted for brightness and contrast in Adobe Photoshop to achieve optimal demarcation of the ultrastructure for presentation in the figures. Criteria for distinguishing a synapse were the presence of (1) parallel pre- and postsynaptic membranes, (2) a postsynaptic density (PSD), and (3) synaptic vesicles at the membrane in the presynaptic terminal. All three criteria had to be fulfilled for the synapse to be counted. Postsynaptic targets were identified based on standard morphological criteria (i.e. size, shape, and internal structures). Spines in the striatum are readily recognizable at the electron microscopic level, as described by Peters et al. (1991). Spines are small and can occasionally be seen originating from a dendritic shaft. Spines contain indistinct filamentous material and the spine apparatus, but tend to lack other organelles, such as mitochondria (Peters et al, 1991). Stereology was used to determine the proportion of labeled and unlabeled terminals forming synapses. Other synaptic features quantified using stereology included the symmetry of the PSD and the postsynaptic target. Neuropil only was quantified, cell bodies were not photographed. Using stereology, an average of 257.2 ± 60.2 synapses were counted in $713.1 \pm 190.1 \mu\text{m}^3$ of the core, and 172 ± 23.8 synapses in $571.8 \pm 95.2 \mu\text{m}^3$ of the shell, per animal. This yielded a total of 1286 synapses in $3565.4 \mu\text{m}^3$ of the core, and 860 synapses in $2858.8 \mu\text{m}^3$ of the shell.

Additionally, single profile sampling was used to quantify PSD measurements and axon terminal cross-sectional area. The length along the postsynaptic membrane and outline of the PSD were traced by hand using NIH ImageJ, and the average thickness was calculated by

dividing the area of the PSD by the measured length (as described in Dosemeci et al, 2001). Axon terminal cross-sectional area was measured by tracing the terminal outline using NIH ImageJ. For PSD and axon terminal measurements, serial images were used to verify that measurements were taken from a full cross-section of the synapse. From each animal, the first 25 synapses of each type were quantified; for categories of synapses that had fewer than 25 per animal, all synapses of that type were quantified. A total of 143–167 synapses were quantified per animal for a grand total of 766 synapses. An outlier analysis of postsynaptic densities identified 3 thicknesses that were either thin but classified as asymmetric, or thick but classified as symmetric. These 3 synapses were removed from the analysis for a total of 763 synapses. Of these, 147 had TH-IR axon terminals.

For stereological analyses, core and shell were analyzed separately. Data from all animals were averaged for each region and statistical tests were performed on average values for each animal ($n=5$). All data sets were assessed for normality with the Kolmogorov-Smirnov test, then the corresponding parametric (paired t -tests) or nonparametric (Wilcoxon matched pairs) tests were performed for within animal comparisons of core vs. shell.

For single profile analyses, core and shell were combined because there were no significant regional differences when core and shell were analyzed separately and compared using paired t -tests. Data for all animals were combined to obtain frequency distributions. All data sets were assessed for normality with the Kolmogorov-Smirnov test. One-way ANOVA with Tukey's Multiple Comparisons post-tests was used for PSD thickness analysis. A Wilcoxon signed rank test was used to compare the cross-sectional areas of all unlabeled ($n=616$) and TH-IR ($n=147$) terminals. All statistical tests were 2-tailed with significance of $p<0.05$.

Results

3.1 Light microscopy

Calbindin immunoreactivity had a higher staining intensity and labeled cell density in the core compared to the shell, providing a clear delineation of the core and shell subregions (Fig. 1). Heterogeneity within the subregions was revealed with calbindin, particularly in more caudal sections of the core where regions of lower calbindin immunoreactivity were observed. The boundary between core and shell however, remained distinct throughout the rostro-caudal extent the NAcc. The rostral pole region described in rat NAcc (Zahm and Brog, 1992; Zahm and Heimer, 1993) was not identifiable in the tree shrew. The dorsal border of the core was indistinguishable from the rest of the dorsal striatum. The ventral boundary of the shell was distinguished by regions largely devoid of calbindin immunoreactivity, corresponding to the rostral extension of the ventral pallidum of the rat, interdigitated by the medial forebrain bundle (Zahm and Brog, 1992; Paxinos and Watson, 1998).

TH immunoreactivity was present throughout the NAcc, with characteristically dense reactivity in the medial shell. The distribution of TH immunoreactivity was uniform throughout the rostrocaudal extent of the NAcc; no gradient was observed in the core or the shell (Fig. 2a–d). In general, the shell appeared to have denser TH immunoreactivity

compared to the core, but the boundary was not as readily distinguishable as with calbindin. TH immunoreactivity was primarily visible in puncta, but was also visible in fibers (Fig. 2e–f). Very few, if any, TH-IR cell bodies were observed in each animal (not shown).

3.2 General ultrastructure

In general, the neuropil ultrastructure consisted of numerous spines, as well as dendrites. These profiles received classic symmetrical and asymmetrical synapses. The average thickness of the PSD of asymmetric synapses was 31.6 ± 4.6 nm, and of symmetric synapses was 17.7 ± 1.3 nm. Occasionally, the PSD of a synapse had an intermediate thickness that was not clearly distinguishable as symmetric or asymmetric in any of the serial micrographs; these were classified separately as “intermediate”. The average PSD thickness of these synapses was 25.1 ± 3.1 nm which differed significantly from both asymmetric and symmetric synapses ($p < 0.0001$, One-way ANOVA with Tukey’s Multiple Comparison test). Intermediate synapses were made almost exclusively by unlabeled axon terminals with spines and were observed sparsely in both the core and shell (7.4% and 3.8% of all synapses, respectively). The axon terminals of these synapses were similar in size and appearance to axon terminals making symmetric and asymmetric synapses.

A total of 1286 axon terminals were quantified in $3565.43 \mu\text{m}^3$ of the core subregion, and 860 axon terminals were quantified in $2858.77 \mu\text{m}^3$ of the shell. The density of synapses in the core and shell, averaged over 5 animals, was 0.37 ± 0.06 per μm^3 and 0.30 ± 0.03 per μm^3 , respectively. Removal of data from the male tree shrew did not significantly affect the results, thus both sexes were included in all analyses.

3.3 TH-IR axon terminals

Labeling was observed in unmyelinated axons and varicosities. In general, TH-IR terminals were smaller than unlabeled axon terminals ($p < 0.0001$, Wilcoxon signed rank test) though they varied in size, ranging from 0.2 – $2.8 \mu\text{m}^2$. The mean TH-IR terminal cross-sectional area was $0.32 \pm 0.38 \mu\text{m}^2$ (compared to $0.47 \pm 0.35 \mu\text{m}^2$ for unlabeled terminals), with areas between 0.1 – $0.2 \mu\text{m}^2$ observed most frequently (Fig. 3a). Large TH-IR terminals ($>1 \mu\text{m}^2$, Fig. 4a) forming synapses were rarely observed. Mitochondria were present in many TH-IR terminals, however other structural features such as microtubules were obscured due to dense DAB precipitate. Of all the axon terminals making synapses, 5.1% ($n=70$) were TH-IR in the core, and 13.4% ($n=108$) in the shell. The density of synapses with TH-IR terminals, averaged over 5 animals, was 0.02 ± 0.01 per μm^3 and 0.04 ± 0.03 per μm^3 in the core and shell, respectively. Although there was a trend for a higher proportion of TH-IR terminals in the shell compared to the core, this trend did not reach significance due to variation between animals.

Many of the TH-IR terminals did not form a synaptic connection or were in close apposition with another profile but failed to exhibit a postsynaptic specialization (Fig. 4b). However, approximately 17.3% and 35.7% of the total TH-IR terminals did form synaptic contact with a proper postsynaptic specialization in the core and shell, respectively. These synapses were primarily symmetric (87.1% and 80.4% in the core and shell, respectively), characterized by a thin PSD and short PSD length (Fig. 4c–e). The distribution of PSD thickness for all

synapses was similar in labeled and unlabeled terminals (Fig 3b). Labeled terminals forming synapses with asymmetric specializations were observed occasionally (Fig. 4f), characterized by a thicker PSD.

3.4 Postsynaptic targets of TH-IR terminals

Within the neuropil, TH-IR terminals synapsed with dendritic spines and dendritic shafts, and occasionally made contact with other axon terminals (Fig. 4). The labeled terminals showed little preference for synapsing on spines versus dendrites in the core: 47.6% synapsed on spines and 49.4% on dendrites, or in the shell: 47.2% synapsed on spines and 45.5% on dendrites (Fig. 5). Synapses onto spines by TH-IR terminals were observed both on the heads and necks of spines (Fig. 4c,e). Those onto spine heads formed convergent input with an unlabeled terminal, where the unlabeled terminals in this arrangement always formed an asymmetric synapse. While labeled and unlabeled terminals were often adjacent to one another, they were rarely observed to make synaptic contact; 3.0% and 7.4% of all labeled terminals made contact with an unlabeled terminal in the core and shell, respectively (Fig. 5). These unlabeled axon terminals receiving contact from TH-IR terminals tended to be large and forming asymmetric synapses on other profiles (Fig. 4g).

Discussion

Rodent models are often used in the study of human striatal disorders, however knowledge that can be gained from these animals is limited by the anatomical differences that exist between the rodent and human striatum. Many diseases with striatal pathology affect the subregions differently, such as in Parkinson's disease and schizophrenia, but the homogenous striatum of the rodent limits our ability to study these subregions individually. While non-human primates provide a more anatomically accurate model than rodents, they are costly and their use in research is complicated by ethical controversy. We suggest that tree shrews could meet the need for an alternative model of the human striatum. Tree shrews are not new to science and have been used as models in other fields of study for decades, establishing them as a practical animal for laboratory use (Fuchs and Corbach-Söhle, 2010).

While the ventral striatum of rodents does have distinguished core and shell subregions of the NAcc which correspond to the core and shell subregions of the human NAcc, studies in rodent and non-human primate have found species differences in the ultrastructural organization of these subregions (Arluison et al, 1984; Bouyer et al, 1984; Voorn et al, 1986; Zahm, 1992; Ikemoto et al, 1996b). The findings of the present study show that the ultrastructure of the tree shrew NAcc reflects that of the primate NAcc and supports their use as an alternative anatomical model of the striatum.

4.1 Synapse types formed by TH-IR terminals

The current study examined the ultrastructural organization and localization of TH in the tree shrew NAcc. As other studies have noted, organization of the NAcc is likely to be more complex than the basic division into core and shell. While the delineation of the core and shell boundary was distinct with calbindin staining in the tree shrew NAcc, heterogeneity within subregions was also observed. The present study examined TH organization at the

level of the core and shell subregions as a goal of this study was to compare the staining patterns to that of other species, and these regions have been described previously across species, including rodent, non-human primate, and human (Rat: Záborsky et al, 1985; Bérubé-Carrière et al, 2012, Non-human primate: Meredith et al, 1996; Brauer et al, 2000, Human: Meredith et al, 1996; Voorn et al, 1996). Regions for the stereological analysis were selected randomly within the core and shell boundaries, thus the further heterogeneity was not selectively targeted for study.

Both the core and shell subregions of the NAcc contained numerous axon terminals labeled with TH, some of which formed proper synapses with unlabeled structures. It is interesting to note that TH-IR axon terminals formed both symmetric and asymmetric synapses. This has been found previously in the NAcc of rat (Arluison et al, 1984; Bouyer et al, 1984; Voorn et al, 1986; Zahm, 1992) and macaque monkeys (Ikemoto et al, 1996b), where both species have a higher proportion of symmetric synapses formed by TH-IR terminals than asymmetric. Dopaminergic terminals have classically been observed to form symmetric synapses which are associated with inhibitory actions, however multiple studies have shown that single dopamine neurons indeed form both synapse types (Hattori et al, 1991; Sulzer et al, 1998). The significance of these neurons forming both types of synapses has been widely discussed and often focuses on evidence that DA or TH colocalizes with markers for excitatory neurotransmitters in dopamine neurons (Sulzer et al, 1998; Kawano et al, 2006; Dal Bo et al, 2004; Yamaguchi et al., 2007) and releases these neurotransmitters synaptically (Sulzer et al, 1998; Joyce and Rayport, 2000; Chuhma et al, 2004; Stuber et al, 2010; Tecuapetla et al, 2010). While the co-release of glutamate from dopaminergic neurons has long been discussed (for reviews, see Sulzer and Rayport, 2000; Trudeau, 2004; Lapish et al, 2007), ultrastructural (Sulzer et al, 1998; Moss et al, 2011; Bérubé-Carrière et al, 2012) and anatomical (Hattori et al, 1991) studies using dual-labeling techniques have shown that the neurotransmitters likely do not colocalize within the same terminals in adult rats. It would therefore be unlikely that an asymmetric PSD of a TH-IR terminal is due to the co-release of glutamate from the same terminal. Colocalization with the CCK-8 peptide has been suggested previously to explain the asymmetric contacts (Arluison et al, 1984), but was not investigated further.

Unique to the tree shrew were synaptic specializations that were indistinguishable as symmetric or asymmetric in any of the serial sections in which they appeared. These synapses, referred to as intermediate in the present study, were formed almost exclusively from unlabeled terminals. As this type of synapse has not been described in the NAcc of other species, it is possible that they are either a characteristic of the tree shrew or an artifact of the preservation and experimental processing. Further studies need to be performed to better classify these synapses, such as axonal degeneration or immuno-electron microscopy with glutamate and GABA, to determine if they are indeed unique synapses and if they arise from extrinsic input or local inhibitory interneurons.

Interaction of TH-IR and unlabeled terminals was observed in the core and shell of the tree shrew NAcc, either indirectly via convergent input onto a common spine, or directly via axoaxonic contact between the TH-IR and unlabeled terminals. This observation is consistent with dopaminergic organization described in previous studies; indirect interaction

has been previously observed in rat and non-human primate (Bouyer et al, 1984; Voorn et al, 1986; Totterdell and Smith, 1989; Sesack and Pickel, 1992; Ikemoto et al, 1996b), and direct interaction in rat (Arluison et al, 1984; Bouyer et al, 1984). This interaction is considered to be the anatomical substrate that allows DA to have a modulatory control of excitatory input to the striatum.

4.2 Comparison with other species

Most interesting about the present study, is how the tree shrew compares to rodents and non-human primates, specifically in characteristics that differ across the species. The core and shell subterritories were readily identifiable in the tree shrew and consistent with the same subregions identified in both rat and primate. The rostral pole of the rodent NAcc which has not been identified in primate NAcc was similarly lacking in the tree shrew. Though the region was not identifiable neurochemically, it remains possible that a region of the anterior NAcc in tree shrew could have similar characteristics to the rostral pole based on the efferent and afferent connections also used to define the region (Zahm and Heimer, 1993).

Several studies have quantified characteristics of dopaminergic ultrastructure in the rodent NAcc (Arluison et al, 1984; Bouyer et al, 1984; Voorn et al, 1986; Zahm, 1992; Bérubé-Carrière et al, 2009), however only one has analyzed the core and shell separately (Zahm, 1992). One study has been done in macaque monkeys (Ikemoto et al, 1996b) which examined only the medial subdivision of the NAcc; a subregion described by Ikemoto and colleagues (Ikemoto et al, 1995) which corresponds to the NAcc shell. Thus, our species comparisons are limited to the data provided in these studies. The percentages of TH-IR terminals making synaptic contact are difficult to compare between tree shrews and rodents due to widely varying findings reported in rodents, ranging from 2.7–69% (Arluison et al, 1984; Bouyer et al, 1984; Voorn et al, 1986; Zahm, 1992; Bérubé-Carrière et al, 2009). Synaptic incidence of TH-IR terminals in tree shrew NAcc shell in the present study was 35.7% compared to 52% in the monkey NAcc shell.

Interestingly, tree shrews and macaques have a similar pattern of postsynaptic target preference in the shell which diverges from that of rodent; labeled terminals in rat showed a significant preference for synapsing more on dendrites than spines, but no preference was observed in tree shrews and macaques (Table 1). No data are present for the NAcc core in nonhuman primates, and neither tree shrew nor rat showed a significant preference for the postsynaptic target of TH-IR terminals in the core (Table 1).

Further differences between rodent and non-human primates have been observed in the proportion of symmetric and asymmetric synapses formed by dopaminergic terminals in the NAcc. Subregional data of this measure were not available for rat, so comparisons were made between combined core and shell data in rat and tree shrew, with data from the medial subdivision in macaques. As mentioned previously, dopaminergic terminals primarily form symmetric synapses in all species, however asymmetric synapses are observed more frequently in macaques than in rats (Bouyer et al, 1984; Voorn et al, 1986; Ikemoto et al, 1996b). Interestingly, the frequency of labeled terminals forming asymmetric synapses in tree shrews was intermediate between rats and macaques (Table 1).

4.3 Potential future uses of tree shrews

The quantifications of the tree shrew ultrastructure from the present study that more closely reflect characteristics of the primate NAcc and diverge from rodent provide further support for tree shrews as an alternative model of the human striatum. The advantage of a more anatomically accurate model of the human striatum is that it would allow for more to be gained from the use of animals in research. It would provide better insight into the question the model is being used to answer, as findings would translate more accurately to the human striatum than is possible with rodents. Its similarity to the primate striatum suggests many potential uses for tree shrews in future research. For example, schizophrenia is understood to be a disorder of disconnection in the brain; however, study of the structural connectivity in rodent models is confounded by anatomical differences that exist between rodents and primates. Future studies could apply the pharmacological, physical, or developmental paradigms currently used to produce rodent models of schizophrenia brain pathology to tree shrews to more accurately study the effects these manipulations have on the wiring of the striatum. Further, tree shrews offer more detailed predictive validity than rodents, as studies in tree shrews could assess the regional effects that drug treatments have within the striatum to potentially disentangle areas of therapeutic action versus side effects. Studies in this manner have been done previously with the treatment of non-human primates, but would now be much more accessible to the research community. The relevance of the NAcc subregions to functions such as reward and motivation suggests the tree shrew would also be beneficial for studies involving drugs of abuse and their effects on the organization of mesolimbic DA projections.

4.4 Limitations

The present study used an antibody against TH, which is a synthesizing enzyme of norepinephrine in addition to dopamine. While TH is an accepted and widely used marker for DA in rodent NAcc studies (Arluison et al, 1984; Bouyer et al, 1984; Zahm, 1992; Bérubé-Carrière et al, 2009), studies in both rodents (Swanson and Hartman, 1975; Hökfelt et al, 1977; Berridge et al, 1997) and primates (Gaspar et al, 1985; Ikemoto et al, 1996b) using an antibody specific to noradrenergic fibers have shown that noradrenergic fibers are present within the NAcc. Noradrenergic innervation of the tree shrew NAcc has not been previously studied thus, it is possible that a small proportion of the TH-IR structures in the present study are noradrenergic rather than dopaminergic.

In rat NAcc, Berridge et al. (1997) describe moderate to dense dopamine beta hydroxylase (DBH) labeling particularly in the dorsomedial and caudal-most regions of the shell and ventral zones, but very few DBH-IR fibers in the NAcc core or rostral shell. In non-human primates and humans, the presence of noradrenaline-IR or DBH-IR fibers are primarily restricted to medial and ventral zones of the NAcc (Gaspar et al, 1985; Ikemoto et al, 1996a). The medial region where DBH-IR is reported to be densest is the region avoided in this study, as described in Section 2.3. Importantly, studies using TH and DA antibodies show similar patterns of staining in the NAcc (Arluison et al, 1984; Bouyer et al, 1984; Voorn et al, 1986; Zahm, 1992; Ciliax et al, 1995; Ikemoto et al, 1996b), and the results of the present study agree with those in studies obtained using an antibody against DA (Voorn et al, 1986; Ikemoto et al, 1996b). Biochemical (Schmidt and Bhatnagar, 1979) and

immunohistochemical (Pickel et al, 1975; Hökfelt et al, 1977) studies, including in the human NAcc (Gaspar et al, 1985), have shown that dopaminergic fibers contain greater amounts of TH than noradrenergic fibers, and that the relatively low TH levels in noradrenergic axon terminals are less readily detected. Thus, previous studies support that TH is a valid marker for studying DA in the NAcc, that the data in the present study represent predominantly dopaminergic organization, and that the deviations in ultrastructure represent species differences rather than variation due to antibody type.

Lastly, it is worth noting that the DAB precipitate in TH-IR terminals was often very dense which could have obscured a synaptic cleft or postsynaptic membrane specialization. The use of serial sections in the present study however, allowed for the examination of profiles in multiple sections, and only those with a clear synaptic cleft and clearly defined PSD were counted as a synapse.

4.5 Conclusions

While comparisons across species cannot be made in the NAcc core due to the lack of data in this region in monkey, comparisons within the shell provide some insight into how the ultrastructural organization of these species compares. Within the NAcc shell, neither tree shrew nor monkey show a preference for the target of synaptic contact by TH-IR terminals, whereas rodent ultrastructure shows a preference for axodendritic contact. Further, when assessing the NAcc as a whole, tree shrews have a higher proportion of TH-IR terminals making asymmetric synaptic contacts than rodents, a feature that has been highlighted as a species difference between monkey and rodents previously (Ikemoto et al, 1996b). Thus, based on organization within the NAcc shell and the proportion of symmetric and asymmetric synapses overall, we suggest that the tree shrew ultrastructure from the present study more closely reflects characteristics of the primate NAcc overall. Together with evidence of neurochemical and morphological similarities of the dorsal striatum (Rice et al, 2011), and similar striatal DA receptor binding patterns (Mijnster et al, 1999), the NAcc ultrastructure from the present study supports that tree shrews may provide an alternative model of the human striatum allowing for further advancement in our understanding of human striatal disorders.

Acknowledgments

The authors would like to acknowledge Dr. Thomas Norton for his generous donation of the tree shrew brain tissue, and Joy Roche for her expert technical support.

Abbreviations

AT	Axon terminal
DAB	Diaminobenzidine
DA	Dopamine
DBH	Dopamine beta hydroxylase
IR	Immunoreactive

NAcc	Nucleus accumbens
PBS	Phosphate buffered saline
TH	Tyrosine hydroxylase

References

- Adkins RM, Honeycutt RL. Molecular phylogeny of the superorder Archonta. *Proc Natl Acad Sci U S A*. 1991; 88:10317–10321. [PubMed: 1658802]
- Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. *Trends Neurosci*. 1989; 12:366–375. [PubMed: 2479133]
- Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*. 1986; 9:357–381. [PubMed: 3085570]
- Arluison M, Dietl M, Thibault J. Ultrastructural morphology of dopaminergic nerve terminals and synapses in the striatum of the rat using tyrosine hydroxylase immunocytochemistry: a topographical study. *Brain Res Bull*. 1984; 13:269–285. [PubMed: 6149794]
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ. Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J Comp Neurol*. 1992; 316:314–347. [PubMed: 1577988]
- Berridge CW, Stratford TL, Foote SL, Kelley AE. Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse*. 1997; 27:230–241. [PubMed: 9329158]
- Bérubé-Carrière N, Guay G, Fortin GM, Kullander K, Olson L, Wallén-Mackenzie Å, Trudeau LE, Descarries L. Ultrastructural characterization of the mesostriatal dopamine innervation in mice, including two mouse lines of conditional VGLUT2 knockout in dopamine neurons. *Eur J Neurosci*. 2012; 35:527–538. [PubMed: 22330100]
- Bérubé-Carrière N, Riad M, Dal Bo G, Lévesque D, Trudeau LE, Descarries L. The dual dopamine-glutamate phenotype of growing mesencephalic neurons regresses in mature rat brain. *J Comp Neurol*. 2009; 517:873–891. [PubMed: 19844994]
- Bouyer JJ, Joh TH, Pickel VM. Ultrastructural localization of tyrosine hydroxylase in rat nucleus accumbens. *J Comp Neurol*. 1984; 227:92–103. [PubMed: 6147361]
- Brauer K, Häusser M, Härtig W, Arendt T. The core-shell dichotomy of nucleus accumbens in the rhesus monkey as revealed by double-immunofluorescence and morphology of cholinergic interneurons. *Brain Res*. 2000; 858:151–162. [PubMed: 10700608]
- Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol*. 1993; 338:255–278. [PubMed: 8308171]
- Chuhma N, Zhang H, Masson J, Zhuang X, Sulzer D, Hen R, Rayport S. Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses. *J Neurosci*. 2004; 24:972–981. [PubMed: 14749442]
- Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI. The dopamine transporter: immunochemical characterization and localization in brain. *J Neurosci*. 1995; 15:1714–1723. [PubMed: 7534339]
- Dal Bo G, St-Gelais F, Danik M, Williams S, Cotton M, Trudeau LE. Dopamine neurons in culture express VGLUT2 explaining their capacity to release glutamate at synapses in addition to dopamine. *J Neurochem*. 2004; 88:1398–1405. [PubMed: 15009640]
- Deniau JM, Menetrey A, Thierry AM. Indirect nucleus accumbens input to the prefrontal cortex via the substantia nigra pars reticulata: a combined anatomical and electrophysiological study in the rat. *Neuroscience*. 1994; 61:533–545. [PubMed: 7526269]
- Dosemeci A, Tao-Cheng JH, Vinade L, Winters CA, Pozzo-Miller L, Reese TS. Glutamate-induced transient modification of the postsynaptic density. *Proc Natl Acad Sci U S A*. 2001; 98:10428–10432. [PubMed: 11517322]

- Fuchs E. Social stress in tree shrews as an animal model of depression: an example of a behavioral model of a CNS disorder. *CNS Spectr.* 2005; 10:182–190. [PubMed: 15744220]
- Fuchs, E.; Corbach-Söhle, S. Tree Shrews. In: Hubrecht, R.; Kirkwood, J., editors. *The UFAW handbook on the care and management of laboratory and other research animals*. eighth edition. Oxford: Wiley-Blackwell; 2010. p. 262-275.
- Gaspar P, Berger B, Alvarez C, Vigny A, Henry JP. Catecholaminergic innervation of the septal area in man: immunocytochemical study using TH and DBH antibodies. *J Comp Neurol.* 1985; 241:12–33. [PubMed: 2865280]
- Geinisman Y, Gunderson HJG, Van Der Zee E, West MJ. Unbiased stereological estimation of the total number of synapses in a brain region. *J Neurocytol.* 1996; 12:805–819. [PubMed: 9023726]
- Gerfen CR, Herkenham M, Thibault J. The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *J Neurosci.* 1987; 7:3915–3934. [PubMed: 2891799]
- Giménez-Amaya JM, McFarland NR, de las Heras S, Haber SN. Organization of thalamic projections to the ventral striatum in the primate. *J Comp Neurol.* 1995; 354:127–149. [PubMed: 7542290]
- Groenewegen HJ. The ventral striatum as an interface between limbic and motor systems. *CNS Spectr.* 2007; 12:887–892. [PubMed: 18163034]
- Groenewegen HJ, Berendse HW. The specificity of the 'nonspecific' midline and intralaminar thalamic nuclei. *Trends Neurosci.* 1994; 17:52–57. [PubMed: 7512768]
- Groenewegen HJ, Berendse HW, Haber SN. Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. *Neuroscience.* 1993; 57:113–142. [PubMed: 8278047]
- Groenewegen HJ, Wright CI, Beijer AV. The nucleus accumbens: gateway for limbic structures to reach the motor system? *Prog Brain Res.* 1996; 107:485–511. [PubMed: 8782538]
- Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phuseolus vulgaris-leucoagglutinin. *Neuroscience.* 1987; 23:103–120. [PubMed: 3683859]
- Haber SN, Fudge JL, McFarland NR. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J Neuroscience.* 2000; 20:2369–2382.
- Haber SN, Kunishio K, Mizobuchi M, Lynd-Balta E. The orbital and medial prefrontal circuit through the primate basal ganglia. *J Neurosci.* 1995; 15:4851–4867. [PubMed: 7623116]
- Haber SN, Lynd E, Klein C, Groenewegen HJ. Topographic organization of the ventral striatal efferent projections in the rhesus monkey: an anterograde tracing study. *J Comp Neurol.* 1990; 293:282–298. [PubMed: 19189717]
- Hattori T, Takada M, Moriizumi T, Van der Kooy D. Single dopaminergic nigrostriatal neurons form two chemically distinct synaptic types: possible transmitter segregation within neurons. *J Comp Neurol.* 1991; 309:391–401. [PubMed: 1717519]
- Heimer L, Alheid GF, de Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS. The accumbens: beyond the core-shell dichotomy. *J Neuropsychiatry Clin Neurosci.* 1997; 9:354–381. [PubMed: 9276840]
- Hökfelt T, Johansson O, Fuxe K, Goldstein M, Park D. Immunohistochemical studies on the localization and distribution of monoamine neuron systems in the rat brain II. Tyrosine hydroxylase in the telencephalon. *Med Biol.* 1977; 55:21–40. [PubMed: 15169]
- Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem.* 1981; 29:577–580. [PubMed: 6166661]
- Ikemoto K, Kitahama K, Maeda T, Satoh K. The distribution of noradrenaline, serotonin and gamma-aminobutyric acid in the monkey nucleus accumbens. *Prog Neuropsychopharmacol Biol Psychiatry.* 1996a; 20:1403–1412. [PubMed: 9004346]
- Ikemoto K, Satoh K, Kitahama K, Geffard M, Maeda T. Electron-microscopic study of dopaminergic structures in the medial subdivision of the monkey nucleus accumbens. *Exp Brain Res.* 1996b; 111:41–50. [PubMed: 8891635]
- Ikemoto K, Satoh K, Maeda T, Fibiger HC. Neurochemical heterogeneity of the primate nucleus accumbens. *Exp Brain Res.* 1995; 104:177–190. [PubMed: 7545584]

- Janecka JE, Miller W, Pringle TH, Wiens F, Zitzmann A, Helgen KM, Springer MS, Murphy WJ. Molecular and genomic data identify the closest living relative of primates. *Science*. 2007; 318:792–794. [PubMed: 17975064]
- Joyce MP, Rayport S. Mesoaccumbens dopamine neuron synapses reconstructed in vitro are glutamatergic. *Neuroscience*. 2000; 99:445–456. [PubMed: 11029537]
- Kawano M, Kawasaki A, Sakata-Haga H, Fukui Y, Kawano H, Nogami H, Hisano S. Particular subpopulations of midbrain and hypothalamic dopamine neurons express vesicular glutamate transporter 2 in the rat brain. *J Comp Neurol*. 2006; 498:581–592. [PubMed: 16917821]
- Koob GF, Nestler EJ. The neurobiology of drug addiction. *J Neuropsychiatry Clin Neurosci*. 1997; 9:482–497. [PubMed: 9276849]
- Kunishio K, Haber SN. Primate cingulostriatal projection: limbic striatal versus sensorimotor striatal input. *J Comp Neurol*. 1994; 350:337–356. [PubMed: 7533796]
- Lapish CC, Kroener S, Durstewitz D, Lavin A, Seamans JK. The ability of the mesocortical dopamine system to operate in distinct temporal modes. *Psychopharmacology (Berl)*. 2007; 91:609–625. [PubMed: 17086392]
- Liu FG, Miyamoto MM, Freire NP, Ong PQ, Tennant MR, Young TS, Gugel KF. Molecular and morphological supertrees for eutherian (placental) mammals. *Science*. 2001; 291:1786–1789. [PubMed: 11230694]
- Lynd-Balta E, Haber SN. The organization of midbrain projections to the ventral striatum in the primate. *Neuroscience*. 1994; 59:609–623. [PubMed: 7516505]
- MacArthur KL, Wu CH, Wu GY. Animal models for the study of hepatitis C virus infection and replication. *World J Gastroenterol*. 2012; 18:2909–2913. [PubMed: 22736914]
- McCollum, LA.; Roberts, RC. Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience; 2012. Ultrastructural characterization of tyrosine hydroxylase in the nucleus accumbens core and shell of the tree shrew. Program No. 895.02. Online
- McCollum LA, Roche JK, Roberts RC. Immunohistochemical localization of enkephalin in the human striatum: a postmortem ultrastructural study. *Synapse*. 2012; 66:204–219. [PubMed: 22034050]
- Meredith GE, Agolia R, Arts MP, Groenewegen HJ, Zahm DS. Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. *Neuroscience*. 1992; 50:149–162. [PubMed: 1383869]
- Meredith GE, Pattiselanno A, Groenewegen HJ, Haber SN. Shell and core in monkey and human nucleus accumbens identified with antibodies to calbindin-D28k. *J Comp Neurol*. 1996; 365:628–639. [PubMed: 8742307]
- Mijnster MJ, Isovich E, Flüge G, Fuchs E. Localization of dopamine receptors in the tree shrew brain using [3H]-SCH23390 and [125I]-epidepride. *Brain Res*. 1999; 841:101–113. [PubMed: 10546993]
- Moss J, Ungless MA, Bolam JP. Dopaminergic axons in different divisions of the adult rat striatal complex do not express vesicular glutamate transporters. *Eur J Neurosci*. 2011; 33:1205–1211. [PubMed: 21375596]
- Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, Teeling E, Ryder OA, Stanhope MJ, de Jong WW, Springer MS. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science*. 2001; 294:2348–2351. [PubMed: 11743200]
- Nauta WJ, Smith GP, Faull RL, Domesick VB. Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience*. 1978; 3:385–401. [PubMed: 683502]
- Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci*. 2000; 23:185–215. [PubMed: 10845063]
- Norton TT. Experimental myopia in tree shrews. *Ciba Found Symp*. 1990; 155:178–199. [PubMed: 2088676]
- O'Donnell P, Greene J, Pabello N, Lewis BL, Grace AA. Modulation of cell firing in the nucleus accumbens. *Ann N Y Acad Sci*. 1999; 877:157–175. [PubMed: 10415649]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 4th edition. San Diego, CA: Academic Press; 1998.

- Perez-Costas, E.; Melendez-Ferro, M.; Roberts, RC. Microscopy techniques and the study of synapses. In: Mendez-Vilas, A.; Diaz, J., editors. *Modern Research and Educational Topics in Microscopy*. 3rd edition. Badajoz, Spain: Formatex; 2007. p. 164-170.
- Perez-Costas E, Melendez-Ferro M, Roberts RC. Basal ganglia pathology in schizophrenia: dopamine connections and anomalies. *J Neurochem*. 2010; 113:287–302. [PubMed: 20089137]
- Peters, A.; Palay, SL.; Webster, HD. *The fine structure of the nervous system: Neurons and their supporting cells*. 3rd ed. New York: Oxford University Press; 1991.
- Pickel VM, Joh TH, Field PM, Becker CG, Reis DJ. Cellular localization of tyrosine hydroxylase by immunohistochemistry. *J Histochem Cytochem*. 1975; 23:1–12. [PubMed: 234988]
- Prensa L, Richard S, Parent A. Chemical anatomy of the human ventral striatum and adjacent basal forebrain structures. *J Comp Neurol*. 2003; 460:345–367. [PubMed: 12692854]
- Rice MW, Roberts RC, Melendez-Ferro M, Perez-Costas E. Neurochemical characterization of the tree shrew dorsal striatum. *Front Neuroanat*. 2011; 5:1–17. [PubMed: 21373368]
- Robbins TW, Everitt BJ. Limbic-striatal memory systems and drug addiction. *Neurobiol Learn Mem*. 2002; 78:625–636. [PubMed: 12559840]
- Schmidt RH, Bhatnagar RK. Assessment of the effects of neonatal subcutaneous 6-hydroxydopamine on noradrenergic and dopaminergic innervation of the cerebral cortex. *Brain Res*. 1979; 166:309–319. [PubMed: 34469]
- Sesack SR, Pickel VM. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol*. 1992; 320:145–160. [PubMed: 1377716]
- Sterio DC. The unbiased estimation of number and size of arbitrary particles using the dissector. *J Microsc*. 1984; 134:127–136. [PubMed: 6737468]
- Stuber GD, Hnasko TS, Britt JP, Edwards RH, Bonci A. Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J Neurosci*. 2010; 30:8229–8233. [PubMed: 20554874]
- Sulzer D, Joyce MP, Lin L, Geldwert D, Haber SN, Hattori T, Rayport S. Dopamine neurons make glutamatergic synapses in vitro. *J Neurosci*. 1998; 18:4588–4602. [PubMed: 9614234]
- Sulzer D, Rayport S. Dale's principle and glutamate corelease from ventral midbrain dopamine neurons. *Amino Acids*. 2000; 19:45–52. [PubMed: 11026472]
- Swanson LW, Hartman BK. The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. *J Comp Neurol*. 1975; 163:467–505. [PubMed: 1100685]
- Tecuapetla F, Patel JC, Xenias H, English D, Tadros I, Shah F, Berlin J, Deisseroth K, Rice ME, Tepper JM, Koos T. Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. *J Neurosci*. 2010; 30:7105–7110. [PubMed: 20484653]
- Totterdell S, Smith AD. Convergence of hippocampal and dopaminergic input onto identified neurons in the nucleus accumbens of the rat. *J Chem Neuroanat*. 1989; 2:285–298. [PubMed: 2572241]
- Trudeau LE. Glutamate co-transmission as an emerging concept in monoamine neuron function. *J Psychiatry Neurosci*. 2004; 29:296–310. [PubMed: 15309046]
- Voorn P, Brady LS, Berendse HW, Richfield EK. Densitometrical analysis of opioid receptor ligand binding in the human striatum—I. Distribution of mu opioid receptor defines shell and core of the ventral striatum. *Neuroscience*. 1996; 75:777–792. [PubMed: 8951872]
- Voorn P, Jorritsma-Byham B, Van Dijk C, Buijs RM. The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscopical study with antibodies against dopamine. *J Comp Neurol*. 1986; 251:84–99. [PubMed: 3760260]
- Wright CI, Groenewegen HJ. Patterns of convergence and segregation in the medial nucleus accumbens of the rat: relationships of prefrontal cortical, midline thalamic, and basal amygdaloid afferents. *J Comp Neurol*. 1995; 361:383–403. [PubMed: 8550887]
- Yamaguchi T, Sheen W, Morales M. Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci*. 2007; 25:106–118. [PubMed: 17241272]
- Záborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M. Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. *Neuroscience*. 1985; 14:427–453. [PubMed: 3887206]

- Zahm DS. An electron microscopic morphometric comparison of tyrosine hydroxylase immunoreactive innervation in the neostriatum and the nucleus accumbens core and shell. *Brain Res.* 1992; 575:341–346. [PubMed: 1349255]
- Zahm DS. Functional-anatomical implications of the nucleus accumbens core and shell subterritories. *Ann N Y Acad Sci.* 1999; 877:113–128. [PubMed: 10415646]
- Zahm DS, Brog JS. On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience.* 1992; 50:751–767. [PubMed: 1448200]
- Zahm DS, Heimer L. Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. *J Comp Neurol.* 1993; 327:220–232. [PubMed: 8425943]

Highlights

- Tree shrews provide a potential anatomical model of the human striatum.
- We analyzed tyrosine hydroxylase in nucleus accumbens using electron microscopy.
- We compared ultrastructural characteristics to published studies in rat and monkey.
- Organization of tree shrew ultrastructure reflects that in primate.
- Findings support tree shrews as an alternative anatomical model of the striatum.

Figure 1.

(a) Immunohistochemical labeling with calbindin (CALB) reveals regions of interest for analysis in (b) tyrosine hydroxylase (TH)-immunolabeled sections. CALB immunolabeling effectively delineates the boundary (arrowheads) between the core (C) and shell (S) subregions of the nucleus accumbens. Pu, putamen; Ca, caudate nucleus; ac, anterior commissure. Scale bars: 1 mm.

Figure 2.

Light microscopy of TH-immunostaining in the NAcc. (a–d) TH-immunostaining appears uniform across the rostrocaudal extent of the NAcc in both the core and shell. Sections are 480 μ m apart. (e–f) TH-immunoreactivity is present in fibers (arrows) and puncta (arrowheads) in the core (e) and shell (f) regions. Scale bars: a–d, 1 mm, e–f, 50 μ m.

Figure 3.

Frequency distribution of axon terminal (AT) cross-sectional area and postsynaptic density (PSD) thickness of all synapses in the core and shell combined. Each bar reflects the total percentage of synapses for that observation, with the shaded portion representing the proportion of TH-IR axon terminals. Data were combined for all animals. **(a)** The cross-sectional areas (μm^2) of all axon terminals making synaptic contact. Mean area for TH-IR terminals $0.32 \pm 0.37 \mu\text{m}^2$ ($n=146$). Mean area for unlabeled terminals $0.47 \pm 0.35 \mu\text{m}^2$ ($n=616$). **(b)** Average PSD thickness (nm) measured as the PSD length divided by the PSD

area. Mean thickness for all TH-IR synapses 20.1 ± 7.4 nm ($n=147$). Mean thickness for all unlabeled terminals 25.2 ± 9.4 nm ($n=617$).

Figure 4.

Electron micrographs from the core and shell. **(a)** TH-IR terminals ranged in size; large terminals (asterisks) were occasionally observed. This terminal has a cross-sectional area of $1.08 \mu\text{m}^2$ and makes an apposition with an unlabeled terminal (arrowhead). **(b)** Apposition (arrowhead) between a TH-IR terminal and a spine which is also receiving an asymmetric synapse from an unlabeled terminal (at). Unlabeled ATs make asymmetric synapses (black arrows) with a spine (sp) and dendrite (den1). A dendrite (den2) receives a symmetric synapse (white arrow). m, mitochondria. **(c)** A TH-IR terminal makes a symmetric synapse (white arrow).

(white arrow) with a spine head (sp) which also receives synapse from an unlabeled axon terminal (at). **(d)** A TH-IR terminal making a symmetric synapse (white arrow) with a dendrite (den). **(e)** A TH-IR terminal makes a symmetric synapse (white arrow) with a spine (sp). **(f)** A TH-IR terminal makes an asymmetric synapse (black arrow) with a spine (sp). **(g)** A TH-IR terminal makes a symmetric contact (white arrow) with an unlabeled axon terminal (at). Scale bars: 0.5 μm .

Figure 5.

The percentage of observations of TH-IR axon terminals that made synaptic contact with dendrites, dendritic spines, or other axon terminals. Synapses were primarily symmetric (sym) but a small proportion of synapses on dendrites and spines had an asymmetric (asym) postsynaptic density. Error bars are standard deviations for postsynaptic target, $n=5$ animals.

Table 1

Percentages and features of TH-IR terminals in the NAcc of different species.

	Tree shrew ^a	Rat	Monkey ^e
Core			
Dendrite	49.4	~38 ^b	NA
Spine	47.5	~54 ^b	NA
Shell			
Dendrite	47.9	~69 ^b	50.7
Spine	41.4	~29 ^b	44.2
NAcc			
Symmetric	81.7	96.0 ^{c,d}	67
Asymmetric	13.3	4.0 ^c ; 1.8 ^d	33

Percentage of TH-IR terminals making synaptic contact with dendrites or spines in the core and shell.

Percentage of TH-IR terminals making symmetric or asymmetric synapses in the NAcc.

^aPresent study.

^bZahm, 1992, percentages estimated from bar graphs.

^cVoorn et al, 1986.

^dBouyer et al, 1984.

^eIkemoto et al, 1996b.

NA, not available.